Release of chromatin protein HMGB1 by necrotic cells triggers inflammation

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High mobility group 1 (HMGB1) protein is both a nuclear factor and a secreted protein. In the cell nucleus it acts as an architectural chromatin-binding factor that bends DNA and promotes protein assembly on specific DNA targets[1,2]. Outside the cell, it binds with high affinity to RAGE (the receptor for advanced glycation end products)[3] and is a potent mediator of inflammation[4–6]. HMGB1 is secreted by activated monocytes and macrophages[4], and is passively released by necrotic or damaged cells[7–9]. Here we report that *Hmgb1*$^{-/-}$ necrotic cells have a greatly reduced ability to promote inflammation, which proves that the release of HMGB1 can signal the demise of a cell to its neighbours. Apoptotic cells do not release HMGB1 even after undergoing secondary necrosis and partial autolysis, and thus fail to promote inflammation even if not cleared promptly by phagocytic cells. In apoptotic cells, HMGB1 is bound firmly to chromatin because of generalized underacetylation of histone and is released in the extracellular medium (promoting inflammation) if chromatin deacetylation is prevented. Thus, cells undergoing apoptosis are programmed to withhold the signal that is broadcast by cells that have been damaged or killed by trauma.

HMGB1 is bound loosely to the chromatin of both interphase and mitotic cells, and is leaked rapidly into the medium when membrane integrity is lost in permeabilized or necrotic cells[7–9]. These results suggest that in living cells HMGB1 associates and dissociates rapidly from chromatin. To prove this, we tagged HMGB1 at its carboxy terminus with green fluorescent protein (GFP), which formed a chimaeric protein that was equivalent to wild-type HMGB1 in enhancing the expression of a HOXD9-responsive reporter gene in transfection assays (ref. 10 and data not shown). HeLa cells expressing the fusion protein were easily detectable by the uniform green fluorescence of their nuclei. Cells undergoing mitosis showed a diffuse cytoplasmic fluorescence, but also a distinct association of HMGB1–GFP with condensed chromosomes that lasted throughout M phase (Fig. 1a and data not shown).

We permeabilized HeLa cells transfected with HMGB1–GFP with Nonidet-P40 (NP-40). Most cells lost their fluorescence after a few seconds, confirming the loose association of HMGB1 with chromatin; however, a few cells retained a bright fluorescence. From the characteristically fragmented appearance of their nuclei, these cells seemed to be apoptotic. We forced HeLa cells to undergo apoptosis by treatment with tumour-necrosis factor-α (TNF-α) and cycloheximide, permeabilized them, and then immunostained them for endogenous, unmodified HMGB1. Although control non-apoptotic cells leaked HMGB1 into the medium (Fig. 1b, d), the protein was retained within the nucleus of apoptotic cells (Fig. 1d). HMGB1 was mostly retained with nuclear remnants even after prolonged incubation and partial autolysis of apoptotic cells, when soluble cytoplasmic proteins such as lactate dehydrogenase leaked into the extracellular medium (Fig. 1e). HMGB1 and HMGB1–GFP also bound tightly to chromatin in HeLa and 3T3 cells that were either induced into apoptosis by treatment with etoposide or H_2O_2, or apoptosing spontaneously in unperturbed cultures (data not shown). By contrast, HMGB1 dissociated from the chromatin of necrotic cells and leaked into the extracellular medium (Fig. 1c).

We used fluorescence loss in photobleaching (FLIP)[11] to quantify the dynamic properties of HMGB1–GFP in single cells. In FLIP, repeated bleaching of the same area leads to fluorescence loss from the rest of the nucleus, with kinetics that are dependent on the overall mobility of the fluorescent protein. If a fraction of the protein pool is at any given time bound to chromatin, the loss of its fluorescence will be slowed. Bleaching of total nuclear HMGB1–GFP was obtained rapidly in living cells (Fig. 2a). By contrast, in HeLa cells that expressed GFP fusions of chromatin proteins HMGN1 and HMGN2, or of transcription factor NF1, fluorescence loss was significantly slower, and in cells expressing GFP–histone H1c fusions fluorescence loss was very limited (ref. 12 and Fig. 2c).

We also assessed the diffusion rate of HMGB1–GFP associated with condensed chromosomes in living HeLa cells during mitosis. The repeated bleaching of cytoplasmic HMGB1–GFP led to a rapid and parallel loss of fluorescence from condensed chromosomes and from the cytoplasm (Fig. 2b, d), which proves unequivocally that

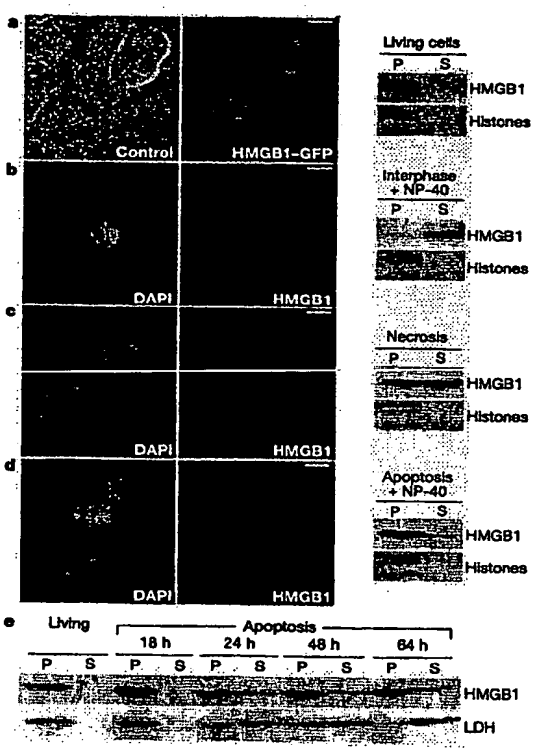

Figure 1 Chromatin association of HMGB1 in living and dead HeLa cells. Both the medium bathing the cells (S) and the cells (P) were analysed by SDS–polyacrylamide gel electrophoresis. Histones were visualized by Coomassie blue staining, HMGB1 by immunoblotting or immunostaining with antibody to HMGB1, DNA by DAPI. Scale bars, 7.5 μm. **a**, Living cells expressing HMGB1–GFP, imaged by differential interference contrast and in green fluorescence. **b**, Interphase cells after permeabilization. **c**, Necrotic cells with no permeabilization. The amount of HMGB1 in the medium was proportional to the number of necrotic cells (about 50%). **d**, Apoptotic cells with permeabilization. **e**, Kinetics of HMGB1 and lactate dehydrogenase (LDH) release from cells undergoing apoptosis and secondary necrosis.

HMGB1 turns over rapidly between its chromatin-bound and soluble states. At the other extreme, HMGB1–GFP seemed to be almost immobile in apoptotic cells (Fig. 2e, g). The blockade of HMGB1 was specific, because the mobility of GFP–HMGN1, GFP–HMGN2, GFP–NF1 or GFP alone was not reduced in apoptotic cells, as compared with living ones (Fig. 2h and data not shown). Thus, chromatin condensation during apoptosis does not impair protein mobility in general.

We used *Hmgb1*$^{-/-}$ cells to test whether the binding of HMGB1 to apoptotic chromatin was caused by alterations in either HMGB1 or nuclei undergoing apoptosis. Embryonic fibroblasts obtained from *Hmgb1*$^{-/-}$ and *Hmgb1*$^{+/+}$ mice[15] were equally susceptible to apoptosis (data not shown), which indicated that the freezing of HMGB1 onto chromatin was a consequence of apoptosis but not a requisite. We treated *Hmgb1*$^{-/-}$ fibroblasts with TNF-α and cycloheximide, and recovered apoptotic cells from the flask by gentle flushing. This cell population, and a control population of non-apoptotic *Hmgb1*$^{-/-}$ fibroblasts, was permeabilized with detergent and exposed to bacterially produced, Cy5-labelled HMGB1. HMGB1 bound to apoptotic nuclei, but not to non-apoptotic ones (Fig. 3a). We verified this result biochemically (Fig. 3b). Permeabilized apoptotic and non-apoptotic *Hmgb1*$^{-/-}$ fibroblasts were incubated with bacterially produced HMGB1 and fractionated through a discontinuous sucrose gradient[9]. Again, HMGB1 associated with the nuclei from apoptotic cells, but not with those from non-apoptotic cells. Together, these findings indicate that on apoptosis chromatin undergoes some chemical or structural transition that makes it susceptible to HMGB1 binding. The nature of

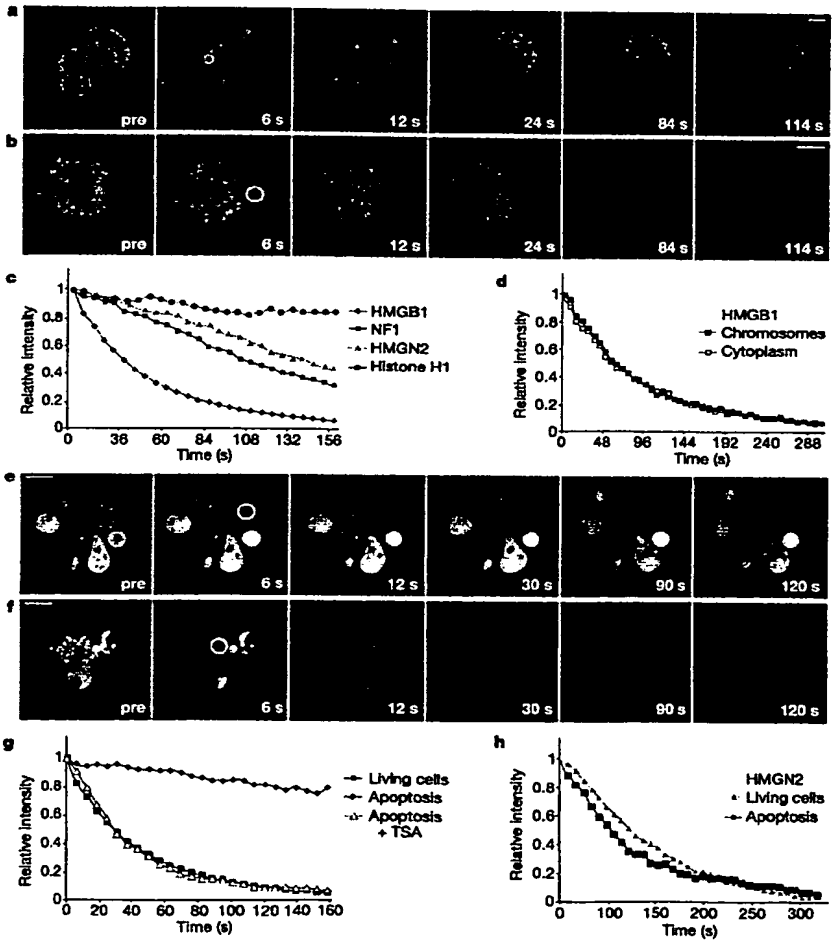

Figure 2 HMGB1 dynamics in living and apoptotic cells. **a**, **c**, FLIP imaging (**a**) and quantification (**c**) of HMGB1–GFP in an interphase cell. The circled area was bleached repeatedly, and cells were imaged between bleach pulses. A neighbouring cell nucleus was not affected. **b**, **d**, FLIP imaging (**b**) and quantification (**d**) on a mitotic cell. Bleaching was executed in the cytoplasm (circle), and quantification was done on a different spot in the cytoplasm or on a spot on the condensed chromosomes. **e**–**g**, FLIP imaging in an apoptotic cell (**e**) and a cell undergoing apoptosis in the presence of 200 ng ml^{-1} TSA (**f**), and their quantification (**g**). **h**, FLIP quantification of GFP–HMGN2 in living and apoptotic cells. Scale bars, 2.3 μm (**a**, **b**) and 3.7 μm (**e**, **f**).

HMGB1 itself, whether endogenous or produced in bacteria, tagged with fluorophores or fused to GFP, is irrelevant.

We next investigated the nature of the chromatin modification that allows the stable binding of HMGB1. Because HMGB1 binds tightly to *in vitro* reconstructed mononucleosomes[9,14,15], we tested whether the fragmentation of chromatin to oligo- and mononucleosomes that occurs in the later stages of apoptosis can provide stable binding sites for HMGB1. HeLa cells were stably transfected with a construct expressing ICAD, the inhibitor of the CAD nuclease that fragments DNA during apoptosis. Although HeLa cells overexpressing ICAD underwent apoptosis, their DNA showed little if any fragmentation (ref. 16 and Fig. 3c). HMGB1 bound equally stably to ICAD-expressing, non-fragmented chromatin and to fragmented chromatin (Fig. 3c and data not shown). DNA fragmentation therefore cannot account for stable HMGB1 binding in apoptosis.

We also tested for alterations in the acetylation status of chromatin. Trichostatin A (TSA), a general deacetylase inhibitor, was added to the medium of HeLa cells just before the induction of apoptosis, which suppressed the binding of HMGB1 on chromatin (Fig. 2f, g). This result suggests that hypoacetylation of one or more chromatin components occurs during apoptosis and favours HMGB1 binding. No difference was seen in the isoelectric point (pI) or the molecular weight pattern of HMGB1 present in apoptotic and non-apoptotic cells (Fig. 3d), which indicates that apoptosis does not change the acetylation status of HMGB1 itself. By contrast, histone H4 from apoptotic chromatin was hypoacetylated in comparison to non-apoptotic chromatin, and H4 hypoacetylation in apoptosis was suppressed by TSA (Fig. 3e and data not shown).

Thus, HMGB1 binding to chromatin depends on the viability of the cell and clearly distinguishes necrotic from apoptotic cells. We therefore reasoned that the differential release of HMGB1 might be exploited as a cue to nearby cells to activate the appropriate responses to unprogrammed and programmed cell death. Unprogrammed death is usually the result of trauma, poisoning or infection, each of which requires prompt reaction, damage containment and/or damage repair. Inflammation is the primary tissue damage response in mammals, and HMGB1 has been reported to be a mediator of inflammation[4–6]. To test directly whether the release of HMGB1 by necrotic cells might be an immediate trigger for an inflammatory response, we challenged wild-type bone marrow cells with *Hmgb1*$^{-/-}$ or wild-type dead fibroblasts. As expected[17], wild-type necrotic cells triggered the production of the proinflammatory cytokine TNF-α, whereas wild-type apoptotic cells were much less effective (Fig. 4a). Significantly, *Hmgb1*$^{-/-}$ necrotic cells were also ineffective in activating monocytes. Purified HMGB1 also elicits TNF-α production in this assay (ref. 6 and data not shown). Thus, HMGB1 is one of the main diffusible signals of necrosis.

The previous experiment does not prove that apoptotic cells escape the inflammatory surveillance owing to retention of HMGB1, because apoptotic cells start to leak cellular components only after several hours and *in vivo* they are routinely cleared by phagocytic cells long before this process (termed 'secondary necrosis') can take place. But we tested whether cells undergoing post-apoptotic, secondary necrosis can promote inflammatory responses in monocytes. Wild-type apoptotic fibroblasts were incubated with for 72 h until most lactate dehydrogenase was released in the extracellular medium. The post-apoptotic cell remnants did not promote a strong inflammatory response in monocytes (Fig. 4b); however, fibroblasts treated with TSA while undergoing apoptosis generated secondarily necrotic cell remnants that promoted inflammation as vigorously as primary necrotic cells that were killed by freeze-thawing.

We could not test whether *Hmgb1*$^{-/-}$ mice have a reduced inflammatory response after tissue necrosis, because these mice survive for only a few hours after birth[13]. To provide evidence in an animal model for the significance of HMGB1 release, we induced massive hepatocyte necrosis in wild-type mice and measured the inflammatory response. Both in humans and rodents, an overdose of the analgesic acetaminophen (AAP; also known as paracetamol) produces large areas of liver necrosis, concomitant with local inflammation, Kupffer cell activation, and the recruitment and sequestration of neutrophils and macrophages into the injured tissue[18,19]. Liver damage and neutrophil sequestration are strictly proportional until most hepatocytes become necrotic between 12

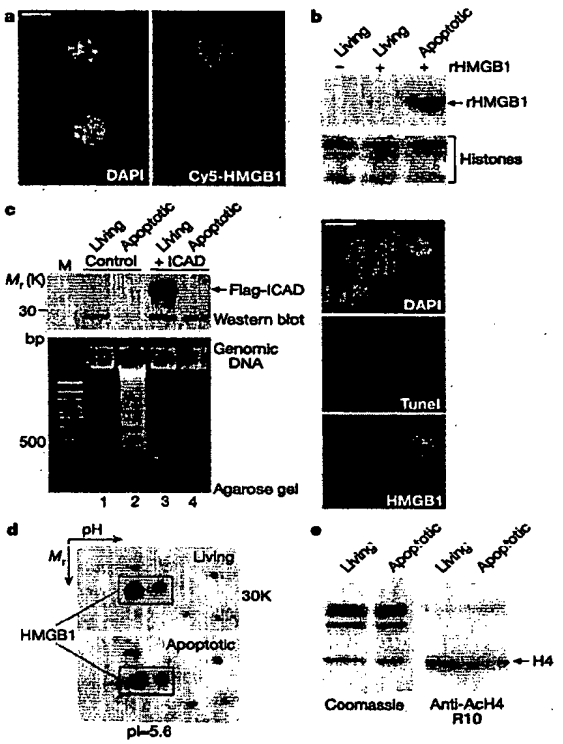

Figure 3 Chromatin changes that occur in apoptosis create binding substrates for HMGB1. Scale bar, 9.5 μm. **a**, **b**, Bacterially produced HMGB1, labelled with Cy5 (**a**) or unlabelled (**b**), binds to the chromatin of apoptotic *Hmgb1*$^{-/-}$ fibroblasts, but not to that of non-apoptotic fibroblasts, as visualized by microscopy (**a**) or western blotting (**b**). Histones were visualized by Coomassie blue staining. **c**, Left, DNA fragmentation is not responsible for HMGB1 binding to apoptotic nuclei. HeLa cells (expressing a tagged form of ICAD, or control) were either induced into apoptosis ('apoptotic', lanes 2 and 4) or mock-treated ('living', lanes 1 and 3). In apoptosis ICAD is cleaved by caspases and loses the Flag tag (western blot, antibody to Flag, lane 4). Agarose gel electrophoresis shows the internucleosomal cleavage of chromosomal DNA in apoptotic wild-type cells (lane 2), and its inhibition in apoptotic ICAD-expressing cells (lane 4). Right, ICAD-expressing apoptotic cells were permeabilized, fixed and stained for DNA, HMGB1 and TUNEL. Whereas all cells are TUNEL-negative, HMGB1 was firmly retained in the nucleus of the cell showing chromatin condensation (top right). **d**, Total extracts from about 5 million living and apoptotic HeLa cells were subjected to two-dimensional electrophoresis and immunoblotted with antibody to HMGB1. Several acetylated forms of HMGB1 are visible, but no difference is detectable between the two samples. **e**, Histone H4 in apoptotic cells is hypoacetylated. Immunoblotting was done with R10 antibody (specific for the acetylated forms of H4).

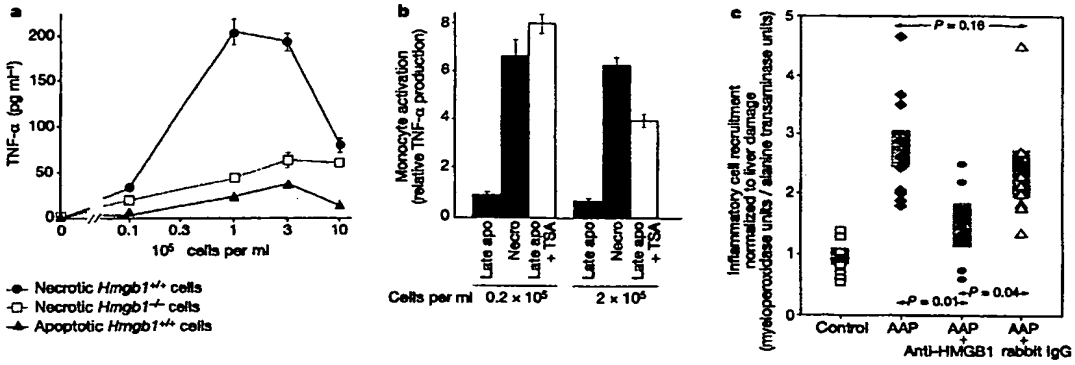

Figure 4 HMGB1 release promotes inflammatory responses. **a**, Necrotic cells lacking HMGB1 do not elicit the production of the pro-inflammatory TNF-α cytokine by monocytes. Bars represent the s.e.m. ($n = 3$). **b**, Apoptotic cells undergoing secondary necrosis and partial autolysis do not promote inflammatory responses unless HMGB1 is mobilized by treatment with TSA. The experiment was repeated three times in duplicate with two different amounts of apoptotic cells to ensure linearity in TNF-α production. Values are normalized to a value of 1 for the amount of TNF-α after challenge with 0.2×10^5 apoptotic cells. **c**, Antibodies to HMGB1 reduce inflammation in liver injured by AAP overdose. Liver injury (alanine transaminase activity in serum) and inflammatory cell recruitment (myeloperoxidase activity in total liver extracts) was assessed after 9 h. MPO/ALT ratios indicate inflammation normalized to liver damage. Each point represents one mouse, the bar indicates the median value, and the grey shading indicates the area included in the mean ± s.e.m. Pairwise comparisons (Mann–Whitney test) between the groups of mice are indicated by the arrows.

and 24 h after AAP poisoning[19]. We administered 300 mg per kg (body weight) AAP with a single intraperitoneal injection to young mice, and estimated liver injury after 9 h by measuring alanine transaminase (ALT) activity in serum, and inflammatory cell sequestration by measuring myeloperoxidase (MPO) activity in total liver extracts. Mice received no AAP ($n = 8$), AAP alone ($n = 10$), AAP plus affinity-purified antibodies to HMGB1 (300 mg per kg; $n = 6$), or AAP plus irrelevant rabbit antibodies (300 mg per kg; $n = 8$). All three groups injected with AAP had higher amounts of ALT than sham-treated controls, but the differences between the three treated groups were not statistically significant. Thus, antibodies do not protect against liver damage, at least at the onset of the inflammatory response.

We used the MPO/ALT ratio to compare inflammatory cell recruitment normalized to the amount of liver damage (Fig. 4c). Antibodies to HMGB1 were effective in reducing inflammation after AAP-induced liver necrosis: these mice showed a significantly reduced MPO/ALT ratio (1.5 ± 0.3) in comparison to both mice injected with AAP alone (2.7 ± 0.3, $P < 0.05$) and mice injected with AAP and pre-immune rabbit IgGs (2.4 ± 0.3, $P < 0.05$). No HMGB1 could be derived from activated monocytes and macrophages in our experiment, because HMGB1 secretion from inflammatory cells requires at least 16 h[4,6]. Thus, HMGB1 acts as an immediate trigger of inflammation, as well as a late mediator of inflammation[4].

In summary, we have shown that the passive release of an abundant chromatin component can serve as a diffusible signal of unprogrammed death, which can be used as a cue to nearby cells. Core histones, although more abundant, would probably not be good signals of necrosis, as they remain anchored to the insoluble chromatin of necrotic cells. Apoptotic cells are not the result of a present and immediate danger and do not trigger inflammation in physiological conditions. They retain nuclear components until cleared by macrophages or nearby cells that act as semiprofessional phagocytes, which they attract and activate by showing 'eat me' signals[20]. But apoptotic cells that escape prompt clearance undergo secondary necrosis leading to an increased amount of nuclear autoantibodies[21], and have been also proposed to have an important pathogenetic role in autoimmune diseases such as lupus[22]. Thus, the retention of HMGB1 by apoptotic cells undergoing secondary necrosis represents an additional safeguard against confusing necrotic and apoptotic cells. □

Methods

Nomenclature

High mobility group proteins have been renamed[23]. High mobility group 1 protein is now officially designated as HMGB1; alternative names are HMG1, amphoterin and p30. HMG-14 and HMG-17 are now HMGN1 and HMGN2.

Constructs and cells

We generated pEGFP–HMGB1 by inserting the coding sequence of the cDNA for rat HMGB1 into pEGFP-N1 (Clontech) using the *EcoRI* and *SacI* restriction sites. Plasmids pEGFP–H1c, pEGFP–NP1, pEGFP–HMGN2 and pEF–flag–mICAD were kindly provided by A. Gunjan, N. Bhattacharyya, R. Hock, M. Bustin and S. Nagata[11,12,16]. HeLa cells and fibroblasts (line VA1, *Hmgb1*$^{+/+}$, and line C1, *Hmgb1*$^{-/-}$) were grown as described[7,13]. We electroporated HeLa cells with pEGFP–HMGB1 and observed them after 18 h. The average amount of HMGB1–GFP in the cell population was between 1 and 3% of HMGB1 (by immunoblotting with antibody to HMGB1). The amount of HMGB1–GFP varied at most tenfold between different cells; care was taken to use cells with a moderate amount of fluorescence for analysis.

We induced apoptosis by treating the cells for 16 h with 2 ng ml^{-1} human TNF-α and 35 μM cycloheximide. Necrosis was induced by treatment for 16 h either with 5 μM ionomycin and 20 μM carbonyl cyanide 3-chlorophenylhydrazone (CCCP), or 6 mM deoxyglucose and 10 mM sodium azide, or by three cycles of freezing and thawing.

Three different clones of HeLa cells stably transfected with pEF–flag–mICAD were analysed by TdT-mediated dUTP nick end labelling (TUNEL; Apoptosis Detection System, Promega), and their chromosomal DNA was extracted and separated by electrophoresis on a 1.5% agarose gel.

Indirect immunofluorescence was done as described[7] using a polyclonal antibody to HMGB1 (Pharmingen) at 1:1,600 dilution, and fluorescein isothiocyanate (FITC)- or tetramethylrhodamine isothiocyanate (TRITC)-conjugated antibodies against rabbit IgG (Boehringer) at 1:300 dilution.

In vivo microscopy and FLIP

Cells were plated and observed in LabTek II chambers (Nalgene) with an Axiovert 135M microscope (Zeiss). We carried out FLIP experiments out on a Leica TCS-SP confocal microscope using the 488-nm excitation line of an Ar laser and detection at 500–575 nm as described[11]. Cells were bleached (in a spot with radius 1 μm, 20 mW nominal output, 200–500 ms) and imaged (at 0.2 mW nominal output) at intervals of 6 s.

Binding of recombinant HMGB1 to chromatin

We treated *Hmgb1*$^{-/-}$ fibroblasts with 2 ng ml^{-1} hTNF-α and 35 μM cycloheximide. After 16 h, apoptotic cells were recovered by gentle flushing of the dish. Ten million apoptotic *Hmgb1*$^{-/-}$ fibroblasts and a control population of non-apoptotic ones were resuspended in 50 μl of PBS containing 0.32 M sucrose, 0.5% NP-40 and 1 μM bacterially produced HMGB1, either fluorescently labelled with Cy5 (Pharmacia) or unlabelled. Average

labelling was 2.3 Cy5 molecules per HMGB1 molecule. After 30 min at room temperature, sample cells were mixed and mounted on slides using Vectashield (Vector Laboratories) containing 1.5 μg ml^{-1} 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), and observed on an Axiophot microscope with a TRITC filter (Carl Zeiss). The two pools of cells incubated with unlabelled HMGB1 were layered onto discontinuous gradients formed by 5 ml of 1.16 M sucrose in PBS and a 6-ml cushion of 2 M sucrose in PBS, and centrifuged at 30,000*g* for 90 min in a SW27 Beckman rotor. We recovered apoptotic and non-apoptotic chromatin that was free of membrane debris from the bottom of the tubes and applied it to a 12% SDS–PAGE gel. The amount of recombinant HMGB1 bound to apoptotic and non-apoptotic chromatin was determined by immunoblotting using an antibody to HMGB1 (Pharmingen) at 1:3,000 dilution. Aliquots of apoptotic and non-apoptotic chromatin were also probed with antibodies to acetyl-histone H4 (R10, a gift from B. Turner), to acetyl-histone H3 (Lys 9, Biolabs) and to acetyl-lysine (Biolabs).

Inflammation assays

To measure TNF-α production *in vitro*, bone marrow was recovered from the hind legs of female C56Bl6 mice, diluted to 5 × 10^6 cells per ml in Optimem and dispensed in 96-well microtitre plates (120 μl per well). Necrotic cells (lysed by three cycles of freeze-thawing) or apoptotic cells were added to the indicated final concentration into the wells and incubated at 37 °C for 18 h. We assayed TNF-α in the supernatant by enzyme-linked immunoabsorbent assay (Quantikine M, R&D Systems). TSA was added at 200 ng ml^{-1} together with TNF-α where indicated, and was washed away before mixing the apoptotic cells with bone marrow cells.

To measure inflammation *in vivo*, 1-day-old mice (weighing 1.1 ± 0.1 g) were injected intraperitoneally with 20 μl of PBS containing 320 μg of acetaminophen (Sigma) and 320 μg of antibodies (Pharmingen BD) where indicated. After 9 h, the mice were analysed for serum ALT activity with the GP-Transaminase kit (Sigma) and for MPO activity in liver extracts as described[24]. We used the non-parametric Mann–Whitney test for statistical analysis of MPO/ALT ratios. Similar results were obtained using Student's *t*-test on the MPO amounts of mice that were paired to minimize the difference in ALT amounts.

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Competing interests statement

The authors declare competing financial interests: details accompany the paper on *Nature*'s website (http://www.nature.com/nature).

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Reciprocal regulation of CD4/CD8 expression by SWI/SNF-like BAF complexes

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Thymic development produces two sub-lineages of T cells expressing either CD4 or CD8 co-receptors that assist antibody production and mediate cell killing, respectively. The mechanisms for mutually exclusive co-receptor expression remain poorly defined[1,2]. We find that mutations in the high mobility group (HMG) domain of BAF57—a DNA-binding subunit of the mammalian SWI/SNF-like chromatin-remodelling BAF complexes—or in the BAF complex ATPase subunit Brg, impair both CD4 silencing and CD8 activation. Brg is haploinsufficient for CD8 activation, but not for CD4 silencing, whereas BAF57 mutations preferentially impair CD4 silencing, pointing to target- and subunit-specific mechanisms of chromatin remodelling. BAF complexes directly bind the CD4 silencer, but the BAF57 HMG domain is dispensable for tethering BAF complexes to the CD4 silencer or other chromatin loci *in vivo*, or for remodelling reconstituted templates *in vitro*[3,4], suggesting that chromatin remodelling *in vivo* requires HMG-dependent DNA bending. These results indicate that BAF complexes contribute to lineage bifurcation by reciprocally regulating lineage-specific genes, reminiscent of the role of the yeast SWI/SNF complex in mediating mating-type switching[5,6].

As germline deletion of several subunits of BAF complexes lead to either early embryonic lethality[7–9] or produce little phenotype[10], we studied the developmental roles of the complexes in the T-cell lineage by inactivating the HMG protein BAF57. The Lck proximal promoter was used to direct expression in transgenic mice of dominant negative mutants of BAF57 lacking the entire amino terminus (BAF57ΔN) or bearing a point mutation (K112I) that disrupts DNA binding[3] (Fig. 1a). Expression of either mutant suppressed endogenous BAF57 expression by up to tenfold apparently through autoregulation (Fig. 1b), indicating that the

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